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Chemical compositions of essential oil and antioxidant activity of dragonhead (*Dracocephalum moldavica*) in sole crop and dragonhead-soybean (*Glycine max*) intercropping system under organic manure and chemical fertilizers

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ABSTRACT

Organic manure can be used as an alternative for chemical fertilizers in sustainable agriculture. In addition, compared with sole cropping systems, intercropping is a promising approach for the crop production due to its lower reliance to chemical fertilizers. In this study, grain yield of soybean and biomass, chemical compositions and antioxidant activity of dragonhead essential oil in sole crop and dragonhead-soybean intercropping system under organic and chemical fertilizers were investigated. Land equivalent ratio (LER) was calculated as well. Results indicated intercropping and application of organic manure, increased soybean grain yield and dragonhead biomass. The LER value for soybean: dragonhead with ratios of 1:1 and1:2 under organic manure was greater than 1, indicating superiority of intercropping versus sol crop systems. GC–MS analysis showed that geranial, geranyl acetate, neral and piperitone were major compounds of dragonhead. The geranial and neral contents were increased in sole cropped dragonhead with application of chemical fertilizer, while the piperitone content was enhanced in sole cropped plants fertilized with organic manure. The highest of geranyl acetate content was observed in intercropped dragonhead plants fertilized with chemical fertilizer. Addition of organic manure lead to increase the antioxidant capacity of dragonhead in intercropped plots. The highest antioxidant activity of dragonhead ($IC_{50} = 1.45\,\mu g mL^{-1}$) was observed in one row of soybean + two rows of dragohead treated with organic manure. Overall, one row of soybean + two rows of dragohead with use of organic manure was more productive and had the highest LER value, antioxidant activity and a large amount of chemical compositions of essential oil. Thus this treatment could be adopted by the medicinal plant growers for appropriate production of dragonhead.

1. Introduction

Legume crops provide an important method of alleviating the constraints related to nitrogen limitations in the soil and enhance crop productivity (Rusinamhodzi et al., 2012). The capacity of legumes to fix atmospheric nitrogen and make it available to other plants (Askegaard and Eriksen, 2007; Fustec et al., 2010) is of particular interest for organic farming (Lithourgidis et al., 2011). Intercropping of legumes with other plants is a practical multi-cropping technique (Li et al., 2006) to increase land-use efficiency and enhance crop yield (Bhatti et al., 2006; Gao et al., 2010). Furthermore, intercropping can suppress weeds (Corre-Hellou et al., 2011), decrease damage caused by pests and diseases (Hauggaard-Nielsen et al., 2001) and improve the quality of the products (Caviglia et al., 2011).

Medicinal plants are reservoirs of useful secondary metabolites for humans. Essential oils and their aromatic constituents are relevant to the production of perfumes, fragrances, food flavoring, pharmaceuticals, as spices and natural food preservatives, for aromatherapy and related medicinal practices (Hadian et al., 2014). Dragonhead (*Dracocephalum moldavica* L.) is an annual herb belonging to the Lamiaceae family (Hussein et al., 2006; Dastmalchi et al., 2007). Extracts and essential oils of this plant are used in the cosmetic, pharmaceutical, food and flavoring industries (Dmitruk and Weryszko-Chmielewska, 2010). Essential oil extracts of dragonhead are reported to possess antioxidant, antimicrobial and antibacterial activities (Dastmalchi et al., 2007).

Most previous studies have focused on ‘the quantity of production of

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legume-non-legume intercropping systems (Lithourgidis et al., 2011; Jannoura et al., 2014; Franco et al., 2015). Few studies have examined intercropping of legumes with medicinal plants. Maeffei and Mucciarelli (2003) found that intercropping of soybeans (*Glycine max*) and peppermint (*Mentha piperita*) increased the menthol content of the peppermint oil. Intercropping of dill (*Anethum graveolens* L.) and common bean (*Phaseolus vulgaris* L.) was found to increase the content of α-phellandrene, β- phellandrene and cryptone in dill oil (Weisani et al., 2015).

Chemical fertilizers are inescapable constituents of current agriculture and have significant effects on world crop production (Siddiqui et al., 2011). Growing concern about the negative impact of chemical fertilizers on the environment and their future cost make it expedient to replace them with organic manure in cultivation practices to increase crop yields (Savci, 2012; Siddiqui et al., 2011).

Application of organic manure can pave the way to replenishing the essential nutrients and improving soil health and crop productivity (Berner et al., 2008; Bajeli et al., 2016). Organic manure could affect plant growth by improving the organic matter content and the soil physical, chemical and biological properties and its carbon, nitrogen, phosphorus and potassium status (Debosz et al., 2002; Ghosh et al., 2004; Kaur et al., 2005).

Studies have shown that the application of organic manure increases the quantity and quality of medicinal plants. Anwar et al. (2005) and Singh et al. (2014) found that the application of organic manure increased the growth, dry matter, oil content, oil yield and methyl chavicol and linalool content of basil (*Ocimum basilicum*). Bajeli et al. (2016) reported the application of farmyard manure alone or combined with vermicompost and poultry manure enhanced yield and menthol content of Japanese mint (*Mentha Arvensise* L.).

The objectives of the present study were: (i) to evaluate the yield and antioxidant activity of dragonhead essential oil as a sole crop and in intercropping systems in response to the application chemical fertilizer and organic manure; (ii) to study the effect of intercropping and organic manure on the chemical compositions of essential oil of dragonhead; (iii) to assess the advantage of dragonhead-soybean intercropping over sole cropping using the land equivalent ratio (LER).

2. Materials and methods

2.1. Experimental design

A factorial experiment was conducted in a randomized complete block design with three replications in 2016 at the Research Farm of Shahrekord University (50°49É, 32°21Ń; 2050 m above sea level). Soybeans and dragonhead seeds were sown in five planting patterns (sole crop dragonhead (D), sole crop soybean (S) and three intercropping ratios of Soybean: Dragonhead (1:2): one row of soybean + two rows of dragonhead, Soybean: Dragonhead (1:1): one row of soybean + one row of dragonhead and Soybean: Dragonhead (2:1): two rows of soybeans + one row of dragonhead) as the first factor using two sources of fertilizer (chemical fertilizer and organic manure) as the second factor.

According to the Koppen climate classification, the climate of region is temperate and cold with warm and dry summers, an average annual precipitation of 316 mm and an average monthly temperature of 12.12 °C. For soil analysis, soil samples were randomly collected from a depth of 0–30 cm at ten points using a soil auger. All soil samples were air-dried in the laboratory for 4 days and then crushed and sieved through a 2 mm sieve. The physical and chemical properties of the soil and the broiler litter were analyzed (Table 1).

In this experiment, the sources of chemical nitrogen and phosphorus were urea and triple superphosphate, respectively. Chemical fertilizers (152 kg ha^{-1} of urea and 141 kg ha^{-1} of triple superphosphate) and organic manure (7.4Mg ha^{-1}) were applied before sowing. The dragonhead and soybean seeds were obtained from Pakan Bazr Company, Isfahan, Iran and Lorestan Agriculture and Natural Resource Research Center, respectively. The plants were sown on 24 May 2016. The soybean and dragonhead were sown at 30 seeds m^{-2} in both the sole and intercropped plots. The sole and intercropped soybeans and dragonhead were sown in the same row. The size of each plot was 2 × 5 m and consisted of 12 rows. The crops were cultivated according to organic agriculture practices with no herbicide or pesticide applications. Weeds were controlled by hand weeding during the growing season.

Table 1
Some physical and chemical properties of soil and used broiler litter.

Property	Soil	Broiler litter
Texture	Clay loam	–
pH	8.09	7.56
EC (dS/m)	0.587	5.57
N (g kg^{-1})	0. 59	19.1
P (g kg^{-1})	0.021	6.9
K (g kg^{-1})	0. 313	12.9

2.2. Determination of soybean grain yield and dragonhead biomass

The dragonhead and soybean were harvested at the complete flowering stage and maturity, respectively. The plants were cut at ground level from each plot with manual shears. For yield determination of the soybean, samples were transferred to the laboratory and kept at 70 °C to dry in an oven for 2 days. After drying, the seed yield was measured in kg ha^{-1}. To determine the biomass of the dragonhead, the samples were dried in the shade. After shade-drying, the biomass was calculated as kg ha^{-1}.

2.3. Nitrogen and phosphorus uptake

The total nitrogen of the dragonhead aerials and soybean grains were determined using the KJeldahl method. The available phosphorus was extracted with sodium bicarbonate and determined by the molybdo-phosphate blue color method using a Pharmacia LKB Novaspec-11 spectrophotometer. The uptake of each nutrient were calculated by multiplying the concentration of nutrient by yield as kg ha^{-1}.

2.4. Isolation of essential oil

At the complete flowering stage, the aerial parts of the dragonhead were harvested, shade-dried and powdered. About 50 g of powdered dragonhead aerial parts were hydrodistilled for 3.5 h using a Clevenger apparatus according to (British Pharmacopoeia, 1988). The samples of essential oils were dehydrated over anhydrous sodium sulphate and stored at 4 °C until gas chromatography–mass spectrometry (GC–MS). The essential oil yield was measured in kg ha^{-1}.

2.5. Gas chromatography

Gas chromatography (GC) was performed using a Thermoquest-Finnigan device equipped with a FID detector and DB-5 column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Helium was used as the carrier gas at a flow rate of 1.1 ml/min. The oven temperature was initiated at 60 °C, then increased from 60 to 250 °C at a rate of 5 °C/ min and kept for 10 min at 250 °C. The injection temperature was 250 °C. The split ratio was adjusted to 100:1.

2.6. Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) analysis was carried out using a Thermoquest-Finnigan device equipped with a DB-5 column (30 m × 0.25 mm i.d.; film thickness 0.25 µm). Helium was used as the carrier gas (flow rate: 1.1 ml/min).The Oven temperature was initiated at 60 °C, then increased from 60 to 250 °C at a rate of 5 °C/min and held for 10 min at 250 °C. The mass spectra were recorded at 70 eV of ionization voltage. The mass range was 40–460 *m/z*. The ionization mode was electron impact (EI) and the temperature of the ionization source was kept at 200 and 250 °C.

2.7. Identification of essential oil components

The chromatographic peaks were identified by the comparison of their Kovats retention indices with those of authentic standards. The calculation of the Kovats index was made based on a linear interpolation of the retention time of the homologous series of *n*-alkanes (C6-C24, Sigma). Data obtained were confirmed by the comparison of the mass spectra of each constituent either with those stored in the Wiley 7.0 and Adams mass spectral-retention index libraries (Adamz, 2007) or with data published in the literature (Yousefzadeh et al., 2013).

2.8. Antioxidant capacity

The antioxidant capacity is most commonly determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity test (Baharfar et al., 2015). Briefly, samples (20 µl of weighted essential oil) were mixed in 100 µl of 0.5 mM DPPH solution in methanol and final volume made to 200 µl. The mixture was shaken and incubated in the dark at room temperature for 15 min. The absorbance of the solutions including blank (without sample) and different concentrations were read at 517 nm in an Awareness Technology, Inc., Elisa reader. Each sample was done in three replications and the data presented as the mean of three values. The inhibition percentage of the samples was calculated as:

$$\text{Inhibition} = \{(A_B\text{-}A_A)/A_B)\} \times 100 \quad (1)$$

where A_A and A_B are the absorbance values of the DPPH radical in the presence of the plant essential oil sample and the control, respectively. The inhibition percentage was plotted versus the sample concentration and 50% of the inhibitory concentration (IC_{50}) of the DPPH values was determined by linear regression analysis.

2.9. Land equivalent ratio

The advantages of dragonhead-soybean intercropping was assessed by calculating the land equivalent ratio (LER) index (Mao et al., 2012) as:

$$LER_{\,=}\,(LER_s\,_+\,LER_d)\;LER_s\,_=\,Y_{si}/Y_s\;LER_d\,_=\,Y_{di}/Y_d \quad (2)$$

where Y_{si} and $Y_s$are the yield of the soybean for intercropping and as the sole crop, respectively, and Y_{di} and Y_d are the yield of dragonhead for intercropping and as the sole crop, respectively.

A LER value greater than 1.0 indicates an intercropping advantage or demonstrates that the interspecific competition is lower than interspecific facilitation, meaning that intercropping resulted in greater land-use efficiency. A LER value of 1.0 indicates that the two intercropped species make similar demands on the same limited resources. A LER value lower than 1.0 indicates mutual antagonism in the intercropping system; thus, a LER value of less than 1.0 has no intercropping advantage and reveals that interspecific competition is higher than interspecific facilitation in the intercropping system (Fetene, 2003; Wahla et al., 2009).

2.10. Statistical analysis

Analysis of variance (ANOVA) was performed as a factorial experiment in a randomized complete block design using SAS software (version 9; SAS Institute; USA). Each data point was the mean of three replications. The mean of the treatments was compared using the least significant difference (LSD) test at the 5% probability level. To evaluate likely similarities among the chemical compositions of the essential oils of the dragonhead from different cropping patterns and fertilization sources, hierarchical cluster analysis was performed using SPSS (ver.16) based on the Ward method.

Table 2
Yield for sole crop and intercrop soybean with dragonhead as response to organic manure and chemical fertilizer.

Cropping pattern	Fertilization source	Yield (kg ha^{-1})	
		Soybean (grain yield)	Dragonhead (aerial yield)
Sole crop of dragonhead	Organic	–	7455 ± 494.3^{a}
Soybean:Dragonhead (1:2)	Organic	2630 ± 42.42^{b}	8317 ± 372.99^{a}
Soybean:Dragonhead (1:1)	Organic	2906 ± 21.21^{a}	7478 ± 221.42^{a}
Soybean:Dragonhead (2:1)	Organic	2810 ± 53.03^{a}	6130 ± 652.66^{b}
Sole crop of soybean	Organic	2640 ± 137.87^{b}	–
Sole crop of dragonhead	Chemical	–	5902 ± 147.50^{b}
Soybean: Dragonhead (1:2)	Chemical	1293 ± 84.85^{f}	5664 ± 216.51^{b}
Soybean:Dragonhead (1:1)	Chemical	1601 + 10.61^{e}	6066 ± 205.8^{b}
Soybean:Dragonhead (2:1)	Chemical	1993 ± 0.005^{d}	5766 ± 890.23^{b}
Sole crop of soybean	Chemical	2375 ± 0.43^{c}	–
Replication	–	NS Ggggg	NS
Cropping pattern (CP)	–	***	*
Fertilization source (FS)	–	***	***
CP × FS	–	***	*
CV (%)	–	2.85	7.46
LSD (p ≤ 0.05)	–	114	862

NS, * and ***: non −significant and significant at $P \le 0.05$ and $P \le 0.001$, respectively. Means within each column with similar letter, are not significantly different ($p \le 0.05$) based on LSD test.

3. Results and discussion

3.1. Yield of dragonhead aerial and soybean grain

The yields of the dragonhead aerials and soybean grains were significantly affected by cropping pattern, fertilization source and interaction between cropping pattern and fertilization source (Table 2). The results indicated the intercropping and application of organic manure increased the yields of both plants. In the sole cropped soybeans, application of manure significantly increased grain yield on an average by 11% in comparison with chemical fertilizer. Different intercropping patterns and the application of manure significantly increased the grain yield of soybean compared with the chemical fertilizer condition (Table 2). The highest grain yield for soybean (for an average of 2906 kg ha^{-1}) was recorded for the Soybean:Dragonhead (1:1) with the application of organic manure. Organic manure application significantly increased the aboveground yield of the sole cropped dragonhead by 26% and intercropped dragonhead under the Soybean:-Dragonhead (1:2) and (1:1) patterns on average by 46% and 23% over chemical fertilizer, respectively. The greatest aboveground yield of dragonhead (on an average of 8317 kg ha^{-1}) was obtained for the Soybean:Dragonhead (1:2) using organic manure; however, there was no significant different under the sole crop and Soybean:Dragonhead (1:1) treated with organic manure.

Intercropping of soybean with dragonhead enhanced yields over values for both sole crops, which indicates that dragonhead-soybean intercropping is superior compared to sole cropping (Table 3). The

Table 3
Nitrogen and phosphorus uptake for sole crop and intercrop soybean with dragonhead as response to organic manure and chemical fertilizer.

Cropping pattern	Fertilization Source	Nitrogen uptake (kg ha^{-1})		Phosphorus uptake (kg ha^{-1})	
		Soybean	Dragonhead	Soybean	Dragonhead
Sole crop of dragonhead	Organic	–	93.80 ± 3.24^{a}	–	17.99 ± 3.42^{ab}
Soybean:Dragonhead (1:2)	Organic	102.84 ± 2.59^{c}	99.78 ± 3.64^{a}	11.43 ± 0.34^{b}	18.27 ± 0.024^{ab}
Soybean:Dragonhead (1:1)	Organic	114.27 ± 5.41^{a}	93.42 ± 0.22^{a}	12.63 ± 0.34^{a}	20.17 ± 0.15^{a}
Soybean:Dragonhead (2:1)	Organic	109.97 ± 1.65^{ab}	71.25 ± 6.75^{bc}	12.08 ± 0.59^{ab}	13.55 ± 2.73^{c}
Sole crop of soybean	Organic	105.93 ± 4.82^{bc}	–	11.50 ± 1.61^{b}	–
Sole crop of dragonhead	Chemical	–	75.25 ± 1.75^{b}	–	13.86 ± 0.32^{c}
Soybean: Dragonhead (1:2)	Chemical	52.16 ± 4.25^{g}	64.55 ± 2.12^{c}	5.43 ± 0.31^{e}	14.17 ± 1.50^{c}
Soybean:Dragonhead (1:1)	Chemical	65.17 ± 0.43^{f}	70.97 ± 2.39^{bc}	6.56 ± 0.43^{d}	13.95 ± 2.73^{c}
Soybean:Dragonhead (2:1)	Chemical	79.55 ± 2.39^{e}	67.16 ± 10.22^{bc}	8.24 ± 0.11^{c}	14.96 ± 2.72^{bc}
Sole crop of soybean	Chemical	93.43 ± 0.37^{d}	–	11.40 ± 0.002^{b}	–
Replication	–	NS	NS	NS	NS
Cropping pattern (CP)	–	**	**	**	NS
Fertilization source (FS)	–	**	**	**	**
CP × FS	–	**	**	**	*
CV (%)		3.64	6.42	6.35	12.32
LSD (p ≤ 0.05)		5.76	8.94	1.09	3.42

NS, * and **: non −significant and significant at $P \le 0.05$ and $P \le 0.01$, respectively.
Means within each column with similar letter, are not significantly different (p ≤ 0.05) based on LSD test.

reasons for the higher yields in such systems are that the intercropped species do not compete for the same growth resources(water, nutrient and light) in a niche and thereby tend to use the available resources in a complementary manner (Lithourgidis et al., 2011). Intercrop components might use the growth resources more efficiently, potentially supporting a greater number of plants and resulting in greater optimum intercrop plant densities than the optimum density for sole crops (Hauggaard-Nielsen et al., 2009). For example, intercropping was shown to improve nitrogen availability through complementarity in nutrient sources from the nitrogen fixation and soil (Tosti et al., 2010). The accumulation of major nutrients such as sulphor, phosphorous, potassium may similarly beincreased by nutrient complementarity in intercrop systems (Hauggaard-Nielsen et al., 2009). The modification of the root architecture due to competition for soil resources could enhance nutrient and water uptake (Li et al., 2011). Previous studies have reported that non-legume–legume intercropping systems can increase yield over their respective monocultures (Wahla et al., 2009; Lithourgidis et al., 2011; Weisani et al., 2015) and the current findings agree with those results.

The application of organic manure enhanced the seed yield of the soybean and the aboveground yield of the dragonhead in sole cropped and intercropped plants. The increase in yield with the application of organic manure could be attributed to higher availability of −nutrients (macro and micro) for longer periods of plant growth and retention of heavy metals in the soil by increasing the cation exchange capacity (Chand et al., 2012), the favorable effects of organic manure on physico-chemical and biological properties of the treated soil (Anwar et al., 2005) and the ability of organic manure to improve the water holding capacity of the soil, thereby augmenting the water supply (Rao et al., 1991). Similar results on Japanese mint, Indian basil and soybean were obtained by Singh et al. (2014); Adeli et al. (2005) and Bajeli et al. (2016), respectively.

3.2. Nitrogen and phosphorus uptake

The nitrogen and phosphorus uptake of the soybean and dragonhead were significantly affected by cropping pattern, fertilization source and interaction between cropping patterns and fertilization source (Table 3). The application of organic manure significantly increased the nitrogen uptake of soybean in the sole crop and intercropping patterns of Soybean: Dragonhead (2:1), (1:1) and (1:2) by 13%, 38%, 75% and 97%, respectively, in comparison with the respective chemical fertilizer (Table 3). Also, the application of organic manure enhanced phosphorus uptake in the sole and intercropped soybeans over chemical fertilizer, but this change was not significant in sole cropped soybeans. The maximum nitrogen uptake (an average of 114.27 kg ha^{-1}) and phosphorus uptake (an average of 12.63 kg ha^{-1}) for soybeans were achieved at Soybean: Dragonhead(1:1) under organic manure; however, there was no significant difference for Soybean: Dragonhead (2:1) under organic manure (Table 3).

The application of organic manure significantly enhanced the nitrogen uptake of the dragonhead in the sole cropping and intercropping patterns of Soybean:Dragonhead (1:2), (1:1) and (2:1) by 24%, 54%, 31% and 6%, respectively, in comparison with the respective chemical fertilizer, but this change was not significant for the Soybean:Dragonhead (2:1) pattern (Table 3). The application of organic manure increased the phosphorus uptake in the sole cropping and intercropping patterns of Soybean:Dragonhead (1:2) and (1:1) by 29%, 28% and 44% compare with the respective chemical fertilizer. The maximum nitrogen uptake (an average of 99.78 kg ha^{-1}) and phosphorus uptake (an average of 20.17 kg ha^{-1}) of dragonhead were recorded for Soybean:Dragonhead (1:2) and (1:1) under organic manure, respectively (Table 3). Nitrogen uptake was higher in soybean plants, while phosphorus uptake was higher in dragonhead plants. The association of non-legume and legumes improved nitrogen and phosphorus uptake through biological fixation of nitrogen and chemical changes in the root zone (Betencourt et al., 2012; Latati et al., 2013).

The non-legume plants required lower amounts of nitrogen to produce the same amount of dry matter, which can be attributed to the higher nitrogen content in the soybean tissue and because most of the nitrogen taken up is through nitrogen fixation (Lithourgidis et al., 2011). Non legume–legume intercropping can encourage rhizosphere acidification through H^+ release by roots of N_2-fixing legumes (Li et al., 2008)This acidification of the rhizosphere could increase dissolution of phosphorus in high pH soils (Hinsinger et al., 2003, Devau et al., 2011), enhancing the availability of soil phosphorus to non-legume plants. Lithourgidis et al. (2011) reported that wheat-pea intercropping enhanced the nitrogen uptake of pea and wheat. Li et al. (2005) reported that intercropping of fava beans and maize increased phosphorus uptake by maize. Th use of organic manure (vermicompost and farmyard manure) enhanced nitrogen and phosphorus uptake of basil (Singh et al., 2014). The application of broiler litter increased the nitrogen phosphorus and uptake of soybean as well (Adeli et al., 2005).

Table 4
Land Equivalent Ratios values of soybean and dragonhead in different intercropping patterns under organic manure and chemical fertilizer.

Intercropping pattern	Fertilization source	$LER_{Soybean}$	$LER_{Daragonhead}$	LER_{Total}
Soybean:Dragonhead (1:2) :1)	Organic	0.33	0.74	1.07
Soybean: Dragonhead (1:1)	Organic	0. 55	0.50	1.05
Soybean: Dragonhead (2: 1)	Organic	0.70	0.27	0.97
Soybean: Dragonhead (1:2)	Chemical	0.18	0.63	0.81
Soybean: Dragonhead (1:1)	Chemical	0.34	0.49	0.83
Soybean: Dragonhead (2:1)	Chemical	0.55	0.32	0.87

3.3. LER of intercropping system

The LER values of different intercropping patterns under organic and chemical fertilizer are presented in Table 4. The LER for Soybean:Dragonhead (1:1) and (1:2) under organic manure was greater than 1. The highest LER (an average of 1.07) was achieved for Soybean:Dragonhead (1:2) under organic manure. The partial LER of soybean (based on grain yield at maturity) increased as the proportion of the dragonhead decreased in the intercropped patterns, whereas the partial LER for the dragonhead (based on aboveground yield) decreased as the proportion of soybean increased under organic manure.

One of the main benefits of the intercropping system is an enhance in yield per unit of land area, expressed as LER (Hauggaard-Nielsen et al., 2009). The LER value for Soybean:Dragonhead (1:1) and (1:2) under organic manure were greater than 1, which shows a yield advantage of intercropping over sole cropping due to the type of land utilization and better use of environmental resources (water, nutrients and light) for plant growth (Banik et al., 2000). The LER values of Soybean:Dragonhead (1:2) and (1:1) were 1.07 and 1.05, which means that 5% to 7% more land area would be required by a monoculture system to achieve a yield equal to that of the intercropping system, indicating greater land use efficiency for intercropping than sole cropping (Midya et al., 2005; Agegnehu et al., 2006). These results agree with the findings of Lithourgidis et al. (2011) in pea (*Pisum arvensis* L.)-triticale (*Triticosecale Wittmack*) and pea-rye (*Secale cereale* L.) intercropping and Weisani et al. (2015) in common bean-dill intercropping.

3.4. Yield and chemical composition of dragonhead essential oil

Cropping pattern had no effect on the essential oil yield of the dragonhead (Table 5). In previous studies, intercropping was shown to enhance the yield of essential oils. Weisani et al. (2015) reported that a common bean-dill intercropping system increased the yield of dill essential oil. Maeffei and Mucciarelli (2003) also observed that intercropping of soybean and peppermint enhanced the yield of essential oil by 50% compared to sole cropping. The application of manure enhanced the yield of dragonhead essential oil by 18.40% compared to chemical fertilizer.

Essential oil is a secondary metabolite and its production is related to higher levels of photosynthetic activity. Increased biomass (Table 2) due to increased availability of nutrients, in particular nitrogen and phosphorus (Table 3), will increase the level of photosynthetic activity, resulting in higher essential oil production in medicinal plants (Croteau et al., 1972). The results of the current study are in agreement with those of Bajeli et al. (2016), who reported that the application of organic manure increased the essential oil yield of Japanese mint. Singh et al. (2014) also observed that the application of farmyard manure

Table 5
Essential oil yield for sole crop and intercrop of dragonhead as response to organic manure and chemical fertilizer.

Treatments	Essential oil yield ($kg\,ha^{-1}$)
Cropping pattern	
Sole dragonhead	21.39^{a}
Soybean:Dragonhead(1:2)	23.52^{a}
Soybean:Dragonhead (1:1)	20.71^{a}
Soybean:Dragonhead (2:1)	21.20^{a}
Fertilization source	
Organic manure	23.53^{a}
Chemical fertilizer	19.87^{b}
Replication	NS
Cropping pattern (CP)	NS
Fertilizer source (FS)	**
CP × FS	NS
CV (%)	10.78

NS and **: non −significant and significant at $P \leq 0.01$, respectively. Means within each column with similar letter, are not significantly different ($p \leq 0.05$) based on LSD test.

enhanced the essential oil yield of basil by 15.75% and 57% in compared to chemical fertilizer and the control, respectively.

The chemical composition of the essential oils of the aerial parts of the dragonhead was established by GC–MS and 19 components in total were identified (Table 6). GC–MS showed that geranial (29.08%–41.52%), geranyl acetate (24.68%–34.8%), neral (21.9%–28.57%) and piperitone (0.59%–7.05%) were the major components (Table 5). Yousefzadeh, et al. (2013) reported that the major components of dragonhead are geraniol, geranial and geranyl acetate. Hussein et al. (2006) and Nikitina et al. (2008) reported that linalool and citral are the major components of dragonhead, respectively.

Variation in the essential oil composition of medicinal and aromatic plants can be attributed to adaptation of geographic origin, particular habitats, ecological factors, agrotechnical approaches and genetic differences (Argyropoulou et al., 2007; Chauhan et al., 2016). The highest neral and geranial contents were achieved in sole-cropped plants with the application of chemical fertilizer, while the highest of content of piperitone (7.05%) was observed in sole-cropped plants with the application of organic manure. Also, in intercropped plants (Soybean:-Dragonhead (1:1)), application of chemical fertilizer increased the content of neryl acetate, geraniol and geranyl acetate in essential oil by 1.99%, 4.85% and 34.8%, respectively. Cluster analysis of the chemical composition of dragonhead essential oils classified them into two groups (Fig. 1). The first group comprised D1: sole crop of dragonhead with application of organic manure, D3: (Soybean:Dragonhead, 1:2) with application of organic manure, D6: (Soybean:Dragonhead, 1:1) with application of chemical fertilizer and D8: (Soybean:Dragonhead, 2:1) with application of chemical fertilizer. This same group was rich in geranyl acetate (28.46%–34.8%) and geranial (29.08%–32.06%). The second group comprised D2: sole crop of dragonhead with application of chemical fertilizer, D4: (Soybean: Dragonhead, 1:2) treated with chemical fertilizer, D5: (Soybean: Dragonhead, 1:1) treated with organic manure and D7: (Soybean: Dragonhead, 2:1) treated with organic manure, with high amounts of geranial (34.56%–41.52%) and geranyl acetate (24.68%–32.74%).

The results of the current study indicate that cropping pattern can influence the chemical composition of the essential oil of dragonhead. Intercropping soybean and dragonhead enhanced the content of minor components and decreased the content of major components. Weisani et al. (2015) found that intercropping of dill and common bean increased the content of α-phellandrene and β- phellandrene while decreasing the content of main components such as dill ether and dill apiole and p-cymene. Peppermint/soybean intercropping increased the content of menthol and decreased the content of menthofuran of peppermint (Maeffei and Mucciarelli, 2003).

Mineral fertilization has been reported to be an agricultural practice

Table 6
Chemical composition of essential oils for sole crop and intercrop of dragonhead as response to organic manure and chemical fertilizer.

		Sole crop		Intercropping					
		(S:D,0:1)		(S:D,1:2)		(S:D,1:1)		(S:D,2:1)	
Compounds	RI	OM	CF	OM	CF	OM	CF	OM	CF
1-Octen-3-ol	977	0.29	0.09	0.3	0.15	0.08	0.23	0.14	0.23
6-Methyl-5-hepten-2-one	984	0.41	0.11	0.43	0.22	0.12	0.32	0.19	0.27
Linalool	1100	0.4	0.14	0.5	0.26	0.17	0.43	0.24	0.37
trans-chrysanthemal	1150	0.18	0.05	0.22	0.07	0.06	0.16	0.08	0.13
Nerol oxide	1155	0.28	0.08	0.3	0.1	0.09	0.24	0.15	0.24
Z-IsoCitral	1164	0.47	0.13	0.36	0.14	0.18	0.12	0.11	0.44
E-IsoCitral	1183	0.74	0.19	0.55	0.25	0.26	0.27	0.2	0.68
Nerol	1234	0.13	0.02	0.1	0.04	Tr	0.13	0.07	0.14
Neral	1248	22.81	28.57	24.84	25.45	26.33	21.9	23.31	21.94
Piperitone	1260	7.05	1.17	5.9	2.88	3.12	4.1	0.59	5.77
Geraniol	1265	3.58	2.31	3	1.47	1.58	4.85	3.83	2.93
Geranial	1279	31.74	41.52	32.06	39.44	34.56	29.14	36.83	29.08
Methyl geranate	1325	0.16	0.04	0.17	0.04	0.04	0.13	0.05	0.12
Neryl acetate	1365	1.95	0.5	1.63	0.56	0.6	1.99	0.96	1.9
Gerayl acetate	1389	28.65	24.68	28.46	28.6	32.41	34.8	32.74	34.4
E-Caryophyllene	1422	0.1	0.03	0.14	0.02	0.04	0.11	0.05	0.13
Germacrene D	1484	0.1	0.02	0.12	0.06	0.05	0.08	0.03	0.16
Unknown	1490	0.27	0.05	0.22	0.02	0.05	0.25	0.07	0.29
Caryophyllene oxide	1587	0.16	0.05	0.14	0.03	0.04	0.15	0.05	0.14
Hexahydrofarnesyl acetone	1847	0.13	0.06	0.19	0.02	0.07	0.2	0.07	0.21
Unknown	2129	0.29	0.07	0.28	0.06	0.07	0.29	0.11	0.32
Total	–	99.33	99.76	99.41	99.8	99.8	99.35	99.69	99.28

RI: Retention indices on the DB-5 column OM: organic manure CF:chemical fertilizer tr:Trace

that can strongly influence essential oil biosynthesis (Alizadeh et al., 2010; Karamanos and Sotiropoulou, 2013). This can be explained by the supply of macronutrients (nitrogen and phosphorus) and has been reported to play an important role in the biosynthesis of some terpenoids (Sell, 2003). The quantity and release rate of nitrogen and phosphorus may affect the quality of the essential oils. Nitrogen plays a key role in the biosynthesis of many organic compounds, including amino acids, proteins, enzymes and nucleic acids. Amino acids and enzymes play a pivotal role in the biosynthesis of numerous compounds that are essential oil constituents (Koeduka et al., 2006). Prasad et al. (2012) also demonstrated that phosphorus fertilization significantly increased the citronellol and 10-epi-y-eudesmol oil content of *P. graveolens*. Pandey et al. (2016) found that application of chemical fertilizer increased the linalool content of basil compared to poultry manure. Pandey and Patra (2015) observed that the application of chemical fertilizer enhanced the isomenthone, citronellol, geraniol, geranyl formate and 10-epi-y-eudesmol content of *Pelargonium graveolens* L'Hér compared to poultry manure.

3.5. Antioxidant capacity of dragonhead

The antioxidant capacity of dragonhead essential oil was significantly affected by cropping pattern ($p \leq 0.001$) and cropping

Table 7
Antioxidant capacity for sole crop and intercrop of dragonhead as response to organic manure and chemical fertilizer.

Cropping pattern	Fertilization source	Antioxidant activity IC_{50}
Sole dragonhead	Organic	5.28 ± 0.13^{a}
Soybean:Dragongead (1:2) :1)	Organic	1.45 ± 0.13^{e}
Soybean: Dragongead (1:1)	Organic	1.87 ± 0.14^{de}
Soybean: Dragongead (2: 1)	Organic	2.01 ± 0.73^{cde}
Sole dragonhead	Chemical	3.19 ± 0.44^{bc}
Soybean: Dragongead (1:2)	Chemical	2.90 ± 0.93^{bcd}
Soybean: Dragongead (1:1)	Chemical	3.03 ± 1.48^{bcd}
Soybean: Dragongead (2:1)	Chemical	3.71 ± 0.28^{b}
Replication	–	NS
Cropping pattern (CP)	–	***
Fertilizer source (FS)	–	NS
CP × FS	–	***
CV (%)		23.35
LSD ($p \leq 0.05$)		1.18

NS and ***: non −significant and significant at $P \leq 0.001$, respectively. Means within each column with similar letter, are not significantly different ($p \leq 0.05$) based on LSD test.

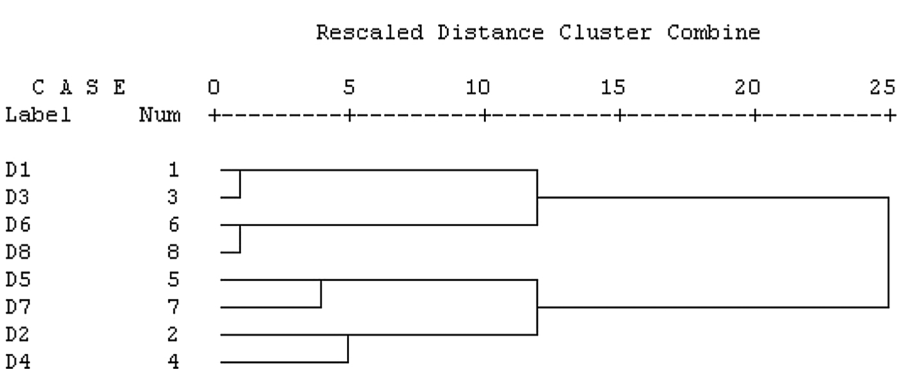

Fig. 1. Classification of eight treatments of dragonhead based on essential oil chemical composition using Ward clustering method. D1, D3, D5 and D7: sole crop of dragonhead, Soybean:Dragonhead (1:2), (1:1) and (2:1) with application organic manure. D2, D4, D6 and D8: sole crop of dragonhead, Soybean:Dragonhead (1:2), (1:1) and (2:1) with application chemical fertilizer.

pattern × fertilization source ($p \leq 0.001$), but this trait was not affected by the fertilization source (Table 7). The antioxidant capacity is the concentration of an essential oil where the DPPH radicals have decreased by half (IC_{50}). In the present study, the IC_{50} values were found to range from 1.45 to 5.28 $\mu g\, mL^{-1}$ (Table 7). Application of organic manure increased the antioxidant capacity of dragonhead in intercropping. In contrast, chemical fertilizer application significantly increased the antioxidant capacity of sole-cropped dragonhead. The treatment Soybean:Dragonhead (1:2) with the application of organic manure showed higher antioxidant potential (the lowest IC_{50} value = 1.45 $\mu g\, mL^{-1}$) than the other treatments, while the lowest antioxidant potential (mean: 5.28 $\mu g\, mL^{-1}$) was observed for the sole-cropping system under organic manure.

An increase in antioxidant capacity among the treatments may be linked to the high quantities of some of the major compounds of the essential oil. An increase in antioxidant activity after the application of organic manure could be caused by the overall promotion of organic manure on the secondary metabolic pathways of the plants (Pandey et al., 2015). Some of the chemical reactions responsible for antioxidant activity in cells involve the macro- and micro-nutrients that are supplied by organic manure (Pandey et al., 2015). Ibrahim et al. (2013) and Pandey et al. (2016) reported that poultry manure increased the antioxidant capacity of basil and *Labisia pumila* compared to chemical fertilizers. No information was available about the mechanism of increase of antioxidant capacity by intercropping. This could be attributed to the effect of intercropping on plant metabolic activity.

4. Conclusion

The findings of the current study indicate that the chemical composition and antioxidant capacity of dragonhead can be significantly affected by environmental and agronomical conditions, including the fertilization source and cropping pattern. Application of organic manure significantly enhanced the essential oil yield of dragonhead. Geranial (29.08%–41.52%), geranyl acetate (24.68%–34.8%), neral (21.9%–28.57%) and piperitone (0.59%–7.05%) were major components. Cluster analysis showed that the chemical composition of essential oils of dragonhead could be classified into two groups. The first group comprised of D1, D3, D6 and D8, all rich in geranyl acetate (28.46%–34.8%) and geranial (29.08%–32.06%). The second group comprised D2, D4, D5 and D7 with high amounts of geranial (34.56%–41.52%) and geranyl acetate (24.68%–32.74%). Intercropping of soybeans and dragonhead with the application of organic manure improved the antioxidant capacity of dragonhead. The LER values for Soybean:Dragonhead (1:1) and (1:2) under organic manure were greater than 1, which indicates that intercropped plants used growth resources more efficiently. Overall, treatment Soybean:Dragonhead (1:1) with the use of organic manure was more productive and had the highest LER and antioxidant activity and a large amount of chemical compositions of essential oil. This treatment could be adopted by farmers for appropriate production of dragonhead.

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